Journal of the Association of Arab Universities for Basic and Applied Sciences (2012) 11, 50-55

حيوية بكتيريا اللبن والتقيم الحسي في الزبادي الحيوي بالقرفة او الثوم والمحضره من حليب الابل والبقر

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الملخص:

أجريت الدراسة الحالية للتحقق من تأثير الخلاصة المائية المحضرة من الثوم (*Allium sativum*) والقرفة (*Cinnamomum verum*) على حيوية بكتيريا اللبن (*Lactobacillus spp.* و *Streptococcus thermophilus*) في الزبادي المحضر من حليب البقر والإبل خلال فترة حفظه في ثلاجة لمدة 21 يوم تحت درجة حرارة 4 درجات مئوية. كما تم خلال هذه الدراسة تقييم الخصائص الحسية للزبادي. تراوحت أعداد .*Lactobacillus* spp في الزبادي الطازج المحضر من حليب البقر في وجود أو عدم وجود الخلاصة المائية للثوم أو القرفة من 10^6 X 1.4 إلى 10^6 X 2.1 cfu/ml في اليوم الأول من البدء في الدراسة. ولم تتغير هذه القيمة بشكل معنوي طوال فترة التخزين المبرد التي استمرت لمدة 21 يوم. أما بالنسبة لأعداد .*Lactobacillus* spp في الزبادي المحضر من حليب الإبل بدون الخلاصة المائية للثوم والقرفة فقد كانت 10^6 X 13.2 cfu/ml في اليوم الأول من البدء في الدراسة، وازدادت أعداد البكتيريا بشكل معنوي، بعد إضافة الخلاصة المائية للقرفة والثوم ، إلى 10^6 X 19.2 و 10^6 X 26.9 cfu/ml على التوالي. في حين أن أعداد هذه البكتيريا انخفضت بشكل خطي في التخزين المبرد بدون الخلاصة المائية. أما بالنسبة لأعداد بكتيريا *Streptococcus thermophilus* في الزبادي المحضر من حليب البقر أو حليب الإبل، سواء بوجود أو في عدم وجود الخلاصة المائية، فقد تراوحت من 2.4 إلى 10^8 X 3.6 cfu/ml مع تزايد الأعداد في اليوم 14 من التخزين المبرد. وفيما يتعلق بالخصائص الحسية فلم توجد اختلافات في الطعم مثل الحموضة أو المرارة أو الحلاوة بين كلا المجموعتين من الزبادي. إلا أن وجود الخلاصة المائية للثوم في زبادي حليب البقر خفضت بشكل معنوي جودة الرائحة إلى 0.7±2.3 مقارنة بزبادي حليب الإبل (1.0±5.5).

Journal of the Association of Arab Universities for Basic and Applied Sciences (2012) **11**, 50–55

University of Bahrain

Journal of the Association of Arab Universities for Basic and Applied Sciences

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ORIGINAL ARTICLE

Viability of lactic acid bacteria and sensory evaluation in *Cinnamomum verum* and *Allium sativum*-bio-yogurts made from camel and cow milk

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Available online 15 December 2011

KEYWORDS

Yogurt;
S. thermophilus;
Lactobacillus spp;
Allium sativum;
Cinnamomum verum

Abstract The present study investigate the effect of herbal water extract prepared from *Allium sativum* and *Cinnamomum verum* on the viability of lactic acid bacteria (*Lactobacillus* spp and *Streptococcus thermophilus*) in cow- and camel-milk yogurts during 21 day refrigerated storage. The organoleptic properties of fresh-yogurts were evaluated. *Lactobacillus* spp count for fresh cow milk-yogurts (0 day) in both present and absent of *C. verum* and *A. sativum* was ranged from 1.4 $\times 10^6$ to 2.1×10^6 cfu/mL. These values were not significantly changed throughout the 21 days of refrigerated storage. *Lactobacillus* spp count in fresh plain camel milk- yogurt was 13.2×10^6 cfu/mL whereas fresh *C. verum*- and *A. sativum*-camel milk- yogurts had higher *Lactobacillus* spp counts (19.2×10^6 and 26.9×10^6 cfu/mL respectively; $p < 0.05$). However, refrigerated storage to 21 days resulted in linear decrease in *Lactobacillus* spp counts. Furthermore, *S. thermophilus* counts in fresh cow- and camel- milk yogurts in either absent or present of *C. verum* or *A. sativum* ranged from 2.4 to 3.6×10^8 cfu/mL and these values increased by day 14 of storage. In organoleptic properties of yogurts no differences were observed in sourness, bitterness, and overall preference scores between the two groups of yogurts. The present of *A. sativum* in cow milk-yogurt reduced the aroma score to (2.3 ± 0.7, $p < 0.05$) compared to camel milk-yogurt (5.5 ± 1.0).

1. Introduction

Recently, the food biotechnology industry has developed a number of commercial products containing a single probiotic strain or bacterial associations of various complexities. Yogurt has been known for its nutraceutical, therapeutic, and probiotic effects (Güler-Akın and Akın, 2007). Also, lactic acid bacteria and its metabolites have shown to play an important role in improving microbiological quality and shelf-life of many fermented food products. Dairy products have long been consumed by consumers and provide a good example of bio-preservation (Zottola et al., 1994).

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Peer review under responsibility of University of Bahrain.
doi:10.1016/j.jaubas.2011.11.001

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Today LAB is a focus of intensive international research for its pivotal role in most fermented foods. Basically, for its ability to produce various anti-microbial compounds promoting probiotic properties (Temmerman et al., 2002) that includes antitumoral activity (De-Vuyst and Degeest, 1999; Østlie et al., 2003), reduction of serum cholesterol (Desmazeaud, 1996; Jackson et al., 2002), alleviation of lactose intolerance (De Vrese et al., 2001), stimulation of the immune system (Isolauri et al., 2001), and stabilization of gut microflora (Gibson et al., 1997). Furthermore, LAB strains synthesize short chain fatty acids, vitamins, and exopolysaccharides (EPS) that are employed in the manufacturing of fermented milk to improve its texture and viscosity (Curk et al., 1996; Ruas-Madiedo et al., 2002). The main technological properties of yogurt bacteria in milk fermentation are acidification, texture enhancement, flavour production, and the final level of lactic acid which is the main product of the metabolic activity of starter cultures. However, the acidification rate during yogurt production depends on the strains and their associations (Beal et al., 1999).

Development of dairy products with new products and flavours has potential health benefits thereby increasing sales and consumers satisfaction. Traditional preparation of yogurt may be beneficial by including other ingredients such as soya protein, vegetables, sweet potato, pumpkin and plum (Joo et al., 2001; Park et al., 2003) to enhance the flavour as well as the nutritional quality (Shori and Baba, 2011). However, traditional medicinal plants such as *Allium sativum* and *Cinnamomum verum* have been proved to provide important therapeutic values. Besides, it is highly aromatic and possesses anti-microbial activities (Harris et al., 2001; Gende et al., 2008) that could affect the yogurts' LAB counts and their organoleptic properties. Therefore, the objective of the present research was to study the survival of LAB in *A. sativum* and *C. verum* yogurts made from cow milk and camel milk and comparison to their respective plain yogurts during 21 day of refrigerated storage and evaluate the organoleptic properties of these yogurts.

2. Materials and methods

2.1. Materials and chemicals

Commercial fresh and pasteurized full cream cow milk (Dutch Lady, Malaysia) and camel milk (Al-Turath, Saudi Arabia) were purchased from supermarket. Camel milk was frozen and used to make yogurt within 2 weeks from the date of pasteurization. The herbs used in the present study were *C. verum* bark purchased from local store in Saudi Arabia and *A. sativum* powder (McCormick, Malaysia). Further supplies incorporated in present study were commercially available yogurt bacteria mixture (Chris-Hansen, Denmark) and probiotic mixture (Bio-Life, Malaysia) in which one capsule contained 5 billion cfu of probiotic bacteria. The agars used in the present study were M.R.S Agar, M17 Agar obtained from Oxoid (Basingstoke, Hampshire, England). Additionally, lactose monohyrate, $C_{12}H_{22}O_{11}{\cdot}H_2O$ was obtained from Systerm.

2.2. Water extraction of herbs

Ten grams of *C. verum* bark and *A. sativum* powder were mixed thoroughly with 100 mL of distilled H_2O. The mixture was incubated overnight in a water bath at 70 °C (Julabo, Model Sw-21c or Haake Model SWD 20) followed by centrifugation (Eppendoft 5804 R; 10000 rpm) for 15 min at 4 °C. The clear supernatants were harvested and used as *C. verum* and *A. sativum* water extracts in the making of herbal yogurts (Behrad et al., 2009).

2.3. Preparation of starter culture

Starter culture for making yogurt was prepared by pre-heating of fresh and pasteurized full cream milk to 41 °C. A mixture of yogurt bacteria consisting of *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* Bb-12, *Lactobacillus casei* LC-01 and *Streptococcus thermophilus* Th-4 in the ratio of 4:4:1:1 and a capsule of probiotic mix containing *L. bulgaricus*, *L. rhamnosus*, *B. infantis* and *B. longum* in the ratio of 1:1:1:1 were mixed thoroughly with the preheated milk prior to an overnight incubation at 41 °C. The yogurt formed was refrigerated (4 °C) and used as starter culture within 7 days (Rashid et al., 2007).

2.4. Preparation of yogurts

C. verum and *A. sativum* yogurts were prepared by mixing 10 mL of each herbal-water extract with 85 mL of pasteurized full cream milk and 5 g of starter culture (Shah, 2003). The mixture was mixed thoroughly followed by incubation at 41 °C. The pH of the mixture was determined every 30 min until the pH of yogurt reached 4.5 by using pH meter (Cyper Scan 510). At that moment, the incubation was terminated by placing the yogurts in ice-bath for 60 min. These yogurts were then placed in the refrigerator for up to 21 days. Control yogurts were prepared using the same procedures except 10 mL of distilled H_2O was used in place of herbal-water extract.

2.5. Microbial viable cell count (VCC) in yogurts

2.5.1. Buffered peptone water

Twenty grams of buffered peptone water was mixed with 1 L distilled water, the mixture was distributed into final tubes followed by autoclaved at 121 °C for 20 min. The pH of media at 25 °C was 7.2 ± 0.2.

2.5.2. Sample preparation

Yogurt samples (1 mL) were individually mixed with 9 mL of 0.15% sterile buffered peptone water. The mixtures were thoroughly stirred and serial dilutions were prepared by using buffered peptone water.

2.5.3. Enumeration of Lactobacillus spp using the pour plate method

Lactobacillus spp was enumerated, particularly, as described by Kailasapathy et al. (2008). MRS agar was prepared by mixing MRS powder with water (62 g/1 L distilled H_2O) and the solution was autoclaved followed by cooling to 45 °C. The melted MRS agar (15 mL) was then placed in a petri dish. Appropriately diluted yogurt (1 mL) was then transferred in the molten MRS agar. The mixture was evenly mixed by gently tilting and swirling the dish. The plates were sealed with parafilm and were left at room temperature to allow the agar to

solidify. Thereafter, the plates were inverted and placed in the incubator (37 °C) for 48 h. Viable *Lactobacillus* spp count was calculated (Sivakumar and Kalaiarasu, 2010) as follows:

$$\text{CFU}^*/\text{mL} = \frac{\text{Number of colonies formed} \times \text{dilution factor of sample}}{1\text{mL of sample}}$$

*CFU: colony forming unit.

2.5.4. Enumeration of S. thermophilus using the spread count method

S. thermophilus was enumerated using M17 agar (Rybka and Kailasapathy, 1995). The M17 agar powder was mixed with water (48.3 g in 950 mL distilled H_2O) and the mixture was sterilized by autoclaving. The molten M17 agar was allowed to cool to 45 °C prior to the addition of sterilized lactose solution (50 mL, 10% w/v). The mixture was dispensed (15 mL) in a petri dish and the molten M17 agar was allowed to solidify at room temperature. Appropriately diluted yogurt (0.1 mL) was placed on the M17 agar and the sample was spread on the surface using a sterile spreader. The plates were incubated in inverted position at 37 °C for 48 h. Viable microbial count (*S. thermophilus*) was calculated (Sivakumar and Kalaiarasu, 2010) as follows:

$$\text{CFU}^*/\text{mL} = \frac{\text{Number of colonies formed} \times \text{dilution factor of sample}}{0.1\text{mL of sample}}$$

*CFU: colony forming unit.

2.6. Organoleptic properties

Organoleptic properties on yogurt were running after 1 day of refrigerated storage. Twelve participants were randomly selected and identified themselves as students and departmental staff. Their age range between 20 and 35 years and were engaged as untrained panels for the sensory evaluation. Each panel was presented with two groups of yogurt (cow-milk and camel-milk yogurts) each group contains three coded yogurt samples (10 mL for each). The first group contained plain-cow-milk yogurt, *A. sativum*-cow-milk yogurt, and *C. verum*-cow-milk yogurt. The second group contained plain-camel-milk yogurt, *A. sativum*-camel-milk yogurt, and *C. verum*-camel-milk yogurt. The evaluation was scored on 1–10 point hedonic scale (1–2 = extremely poor, 3–4 = poor, 5–6 = fair, 7–8 = good, 9–10 = excellent) according to taste (sour, sweet, and bitter), aroma and overall preference.

2.7. Statistical analysis

The experiment was designed according to a 2×3 factorial design. All experiments were performed in three batches ($n = 3$) and the average was taken. Data were expressed as mean ± standard error using one-way ANOVA by SPSS® version 17.0. Means were compared using Duncan's multiple range tests, and statistical significance was standard by ANOVA at $p < 0.05$.

3. Results

3.1. Survival of lactic acid bacteria into plain and herbal yogurts

*3.1.1. Bacteria counts of **Lactobacillus** spp*

Lactobacillus spp counts were 1.4×10^6, 2.1×10^6, and 1.7×10^6 cfu/mL for fresh plain-, *C. verum*- and *A. sativum*-cow-milk yogurts, respectively (Fig. 1). *Lactobacillus* spp counts increased to about 2.3×10^6 cfu/mL for all three yogurts by day 7 of storage with significant effect ($p < 0.05$) seen in plain-cow-milk yogurt. *Lactobacillus* spp counts in all three yogurts were almost similar during the 14 days of storage but the viable cell counts reduced gradually to 1.4×10^6 cfu/mL for plain- and *A. sativum*-cow-milk yogurts and 1.7×10^6 cfu/mL for *C. verum*-cow-milk yogurt by day 21 of storage.

In contrast, *Lactobacillus* spp counts in fresh camel-milk yogurts were about tenfold higher than in fresh cow-milk yogurts (Figs. 1 and 2). The viable cell count in plain-camel-milk yogurt was 13.2×10^6 cfu/mL; however, the addition of *C. verum* and *A. sativum* increased ($p < 0.05$) the counts to 19.2×10^6 and 26.9×10^6 cfu/mL, respectively. There were small decreases in *Lactobacillus* spp counts in yogurts ($p < 0.05$, for plain-camel-milk yogurt) after 7 days refrigerated storage. However, pronounced reduction in *Lactobacillus* spp counts occurred in plain- and *A. sativum*-camel-milk yogurts over the 14 days with lowest count being 1.3×10^6 and 1.7×10^6 cfu/mL, respectively, on day 21 of storage. *Lactobacillus* spp counts in *C. verum*-camel-milk yogurts reduced slowly during this period resulting in highest counts (4.3×10^6 cfu/mL) amongst the three types of camel-milk yogurts on day 21 of storage.

3.1.2. Bacteria counts of Streptococcus thermophilus

S. thermophilus counts in fresh cow- and camel-milk yogurts ranged $2.0–3.0 \times 10^8$ cfu/mL (Figs. 3 and 4). The viable cell counts increased with refrigerated storage to similar values for all plain-, *A. sativum*- and *C. verum*-cow-milk yogurts and reached the highest counts 4.30×10^8, 4.90×10^8 and 5.30×10^8 cfu/mL, respectively, by day 14 of storage; followed by a small reduction to 3.7×10^8, 4.50×10^8 and 4.70×10^8 cfu/mL, respectively, by day 21 of storage. In comparison, the viable cell counts in camel-milk yogurts increased almost twofold higher by day 14 of storage (9.5×10^8, 11.7×10^8 and 9.9×10^8 cfu/mL) for plain-, *A. sativum*- and *C. verum*-camel-milk yogurts, respectively. Extension of storage to day 21 resulted in a decrease in viable S. *thermophilus* counts to 7.0×10^8 cfu/mL for both plain- and *C. verum*-camel-milk yogurts but not for *A. sativum* camel-milk yogurt which was

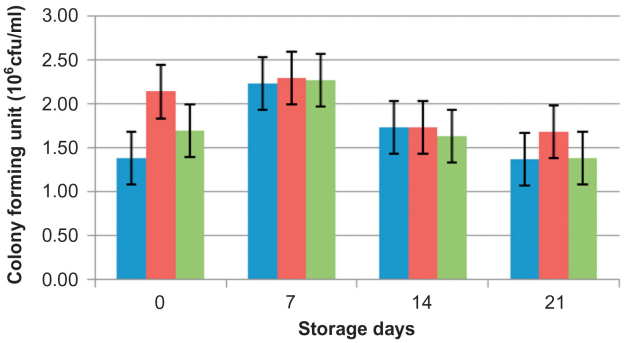

Figure 1 Changes in bacterial counts of *Lactobacillus* spp (10^6 cfu/mL) during 21 day refrigerated storage (4 °C). ■ *C. verum*-cow-milk yogurt, ■ *A. sativum*-cow-milk yogurt versus control ■ plain-cow-milk yogurt. Values are presented as mean ± SEM ($n = 3$). For *C. verum*-cow-milk yogurt (0 day) ANOVA showed a significant effect at 5% level.

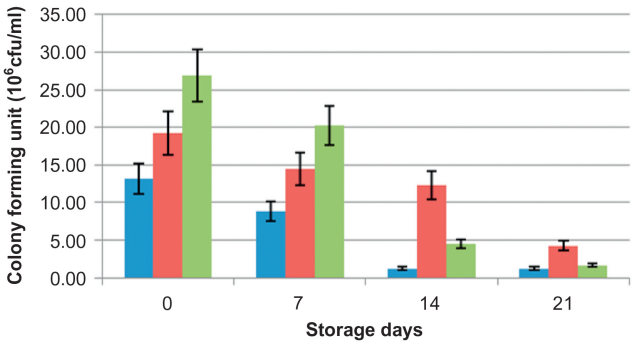

Figure 2 Changes in bacterial counts of *Lactobacillus* spp (10^6 cfu/mL) during 21 day refrigerated storage (4 °C). ■ *C. verum*-camel-milk yogurt, ■ *A. sativum*-camel-milk yogurt versus control ■ plain-camel-milk yogurt. Values are presented as mean ± SEM (n = 3). For both cow- and camel-milk yogurts in the presence of *C. verum* and *A. sativum* ANOVA showed a significant effect at 5% level during all periods of storage.

12.5×10^8 cfu/mL. The addition of *A. sativum*- and *C. verum* in cow-milk yogurts showed no significant effect on the survival rate of *S. thermophilus* compared to their control (plain yogurt). However, the addition of *A. sativum* in camel-milk yogurts showed increased ($p < 0.05$) in *S. thermophilus* counts on 7 and 21 days compared to its control while, there was no effect on *S. thermophilus* counts in the presence of *C. verum*.

3.2. Organoleptic properties of stored yogurts

The sensory evaluations of cow- and camel-milk yogurts were shown in Table 1. No differences were observed in sourness, bitterness, and overall preference scores between the two groups of yogurts, both in the absence and presence of *A. sativum* or *C. verum*. The plain yogurt sweetness score was greater for cow-milk yogurt than the camel-milk yogurt. However, the presence of *A. sativum* and *C. verum* effects on sweetness was reduced in cow-milk yogurt, but increased in camel-milk yogurt. The aroma score was slightly greater in cow-milk yogurt (6.1 ± 1.8) than in camel-milk yogurt (5.4 ± 1.4). The presence of *A. sativum* reduced aroma score in cow-milk

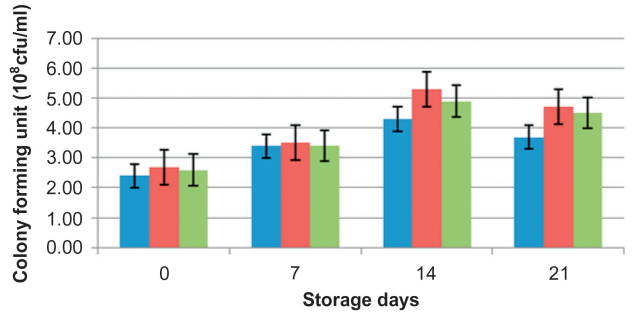

Figure 3 Changes in bacteria counts of *Streptococcus thermophilus* (10^8 cfu/mL) during 21 day refrigerated storage (4 °C). ■ *C. verum*-cow-milk yogurt, ■ *A. sativum*-cow-milk yogurt versus control ■ plain-cow-milk yogurt. Values are presented as mean ± SEM (n = 3). For all treated yogurt ANOVA showed no significant effect at 5% level.

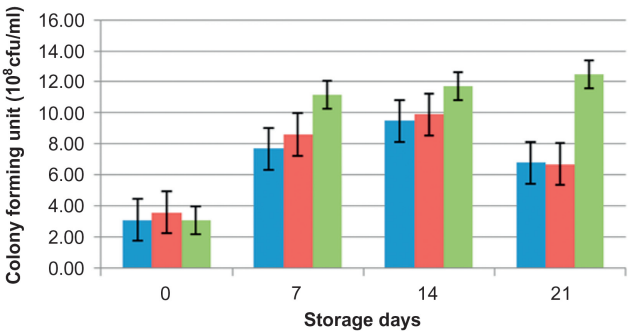

Figure 4 Changes in bacteria counts of *Streptococcus thermophilus* (10^8 cfu/mL) during 21 day refrigerated storage (4 °C). ■ *C. verum*-camel milk yogurt ■ *A. sativum*-camel-milk yogurt versus control ■ plain-camel-milk yogurt. Values are presented as mean ± SEM (n = 3). For *A. sativum*-camel-milk yogurt at 7 and 21 days ANOVA showed a significant effect at 5% level.

yogurt (2.3 ± 0.7, $p < 0.05$) contrary to camel-milk yogurt (5.5 ± 1.0).

4. Discussion

In this present study, the viable counts of *Lactobacillus* spp for both types of yogurts confirmed to reduce during refrigerated storage (by day 14 for cow-milk yogurts and day 7 for camel-milk yogurts). This result was in agreement with previous study that found refrigerated storage decreased the viable counts of *Lactobacillus* spp significantly by the 14th day of refrigerated storage (Shah and Ravula, 2001; Haynes and Playne, 2002; Kailasapathy and Sultana, 2003; Laniewska-Trokenheim et al., 2010). Additionally, the reduction of *Lactobacillus* spp counts could be associated with the post-acidification of yogurt which causes a further reduction in pH values (Shah, 2000; Omer and Eltinay, 2009; Eissa et al., 2010). However, in this present study, the pH of cow-milk yogurts reduced at faster rates than camel-milk yogurts during refrigerated storage (data not shown). Thus, the more rapid reduction of *Lactobacillus* spp counts in all camel-milk yogurts than cow-milk yogurts (Figs. 1 and 2) could be attributed to the higher antibacterial properties of camel milk than cow milk (El Agamy et al., 1992).

Conversely, the increase in the viability of *S. thermophilus* in both cow-milk and camel-milk yogurts throughout the first 14 days of refrigerated storage was in agreement with other previous studies (Birollo et al., 2000). To the best knowledge of the researchers, this is the first simultaneous report on the survival of both *Lactobacillus* spp and *S. thermophilus* during refrigerated storage which showed higher survival percentage of these bacteria in cow-milk than in camel-milk yogurts. Furthermore, the significant drop in the viable cell counts of *S. thermophilus* by day 21 of storage in both cow-milk and camel-milk yogurts may be attributed to the accumulation of organic acids (Østlie et al., 2003). However, the sustained survival of *S. thermophilus* in *A. sativum*-camel-milk yogurt indicated a positive effect of its addition into camel milk during yogurt preparation. The reason is not clear. Thus, further studies are required.

Viable LAB was higher in camel-milk than in cow-milk yogurts and this may be partly explained by the higher free amino

Table 1 Mean taste panel scores for herbal yogurts versus control (plain yogurt) made from cow and camel milk.

	P-cow-milk Y[a,b,c]	AS-cow-milk Y[a,b,c]	CV-cow-milk Y[a,b,c]	P-camel-milk Y[a,b,c]	AS-camel-milk Y[a,b,c]	CV-camel-milk Y[a,b,c]
Sourness	5.83 ± 1.95	6.42 ± 1.73	5.67 ± 1.72	6.42 ± 1.93	5.92 ± 1.68	5.67 ± 1.15
Sweetness	4.83 ± 1.19	3.42 ± 1.16	3.58 ± 1.83	3.83 ± 1.75	4.92 ± 1.38	4.83 ± 1.27
Bitterness	2.92 ± 1.51	3.08 ± 1.88	2.42 ± 1.24	2.42 ± 1.31	2.17 ± 1.27	2.83 ± 1.64
Aroma	6.08 ± 1.83	2.33 ± 0.65	6.33 ± 1.50	5.42 ± 1.44	5.50 ± 1.00	5.75 ± 1.48
Overall preference	6.50 ± 1.45	5.08 ± 1.24	5.92 ± 1.56	6.17 ± 1.64	5.75 ± 1.71	6.00 ± 1.21

[a] P = plain, AS = *A. sativum*, CV = *C. verum* and Y = yogurt.
[b] First group (AS-cow-milk Y, CV-cow-milk Y versus P-cow-milk Y), second group (AS-camel-milk Y, CV-camel-milk Y versus P-camel-milk Y).
[c] Values are presented as mean ± SEM, $n = 12$. Significant for aroma at AS-cow-milk Y.

acids in camel milk than in cow milk (Mehaia and Al-Kanhal, 1992), and higher milk protein proteolysis by *L. delbrueckii* spp *bulgaricus* in camel milk than in cow milk (Abu-Tarboush, 1996). Both factors contribute to apparent higher digestibility of camel milk than cow milk which readily supported growth and metabolism of LAB during fermentation and refrigerated storage. Therefore, the higher 'mortality' of *Lactobacillus* spp in camel-milk yogurts during refrigerated storage may not affect its functional values because the viable cell counts of LAB on the 3rd week of storage in camel-milk yogurts were still higher than those in the 2nd week of storage for cow-milk yogurts.

Furthermore, in this present study, the decrease in the aroma score for *A. sativum*-camel-milk yogurt could be explained via study conducted by Hansanugrum and Barringer (2010) which found that milk proved effective in the deodorization of AMS (allyl methyl sulphide) latter identified as responsible for the 'garlic odour' (Block, 2010). Moreover, the significant higher aroma score for *A. sativum*-camel-milk yogurt than *A. sativm*-cow-milk yogurt suggested that camel milk was more effective in the deodorization of AMS than cow milk.

5. Conclusion

A. sativum and *C. verum* enhanced *Lactobacillus* spp counts more in camel-milk yogurts than in cow-milk yogurts with respect to growth during fermentation except they could not sustain *Lactobacillus* spp survival in camel-milk yogurts during refrigerated storage. However, these herbs did not affect *S. thermophilus* counts in camel- and cow-milk yogurts both during fermentation and refrigerated storage. The addition of *A. sativum* and *C. verum* did not affect the organoleptic properties of cow- and camel-milk yogurts although *A. sativum* may reduce the aroma score in the former but not in the latter.

Acknowledgement

We acknowledge the financial support of University of Malaya Research Grant (RG023-090BIO).

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